ANTHOCYANIN PROFILE AND ANTIOXIDANT ACTIVITY FROM 24 GRAPE VARIETIES CULTIVATED IN TWO PORTUGUESE WINE REGIONS

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Abstract

Aims: The purpose of this work was to evaluate the general phenolic composition and the anthocyanin profile of 24 grape varieties from two Portuguese wine regions as well as their antioxidant activity in the different grape berry fractions (skins, pulps and seeds).

Methods and results: Individual anthocyanin composition of grape skin extracts was analyzed by HPLC, whereas total antioxidant activity was evaluated by two methods: DPPH and ABTS. In general, a high variability was found among the different autochthonous and non-autochthonous grape varieties in relation to the polyphenolic compounds analyzed, especially the individual anthocyanins. The individual anthocyanins in grape skin extracts were mainly malvidin (1.40-7.09 mg/g of skin), in particular malvidin-3-glucoside (0.62-6.09 mg/g of skin). The highest antioxidant activity was consistently detected in the seed extracts; however, it was not possible to establish a clear difference among the grape varieties analyzed.

Conclusion: High variability in polyphenolic content, individual anthocyanin composition and antioxidant activity was found among the diverse autochthonous and non-autochthonous grape varieties studied. Seeds showed the highest antioxidant activity, followed by skin and pulp, irrespective of the grape variety.

Significance and impact of the study: Most vineyards in Portugal grow Portuguese cultivars of *Vitis Vinifera* L. and other cultivars grown worldwide. The phenolic compounds and antioxidant activity of these grape cultivars have never been characterized under the environmental conditions of the *Douro* and *Dão* regions. The variability in phenolic content among the grape varieties studied confirms the hypothesis that genetic factors have an important role in the biosynthesis of these compounds and, consequently, in the antioxidant activity of grapes.

Key words: antioxidant activity, individual anthocyanins, red grapes, pulp, seed, skin

Résumé

Objectifs : Le but de ce travail était d'évaluer la composition phénolique générale et le profil d'anthocyanes de 24 cépages de deux régions viticoles portugaises ainsi que leur activité antioxydante dans les différentes fractions de baie de raisin (pellicules, pulpes et pépins).

Méthodes et résultats : Les anthocyanes obtenues à partir d'extraits de pellicules de raisin ont été analysées par HPLC tandis que l'activité antioxydante totale a été évaluée par deux méthodes : DPPH et ABTS. En général, une grande variabilité a été observée parmi les différentes variétés de raisins autochtones et non autochtones en ce qui concerne les composés polyphénoliques analysés comme les anthocyanes. Dans les extraits de pellicules de raisin, les anthocyanes étaient principalement la malvidine (1.40-7.09 mg/g de pellicule), et en particulier la malvidine-3-glucoside (0.62-6.09 mg/g de pellicule). Dans tous les cépages étudiés, l'activité antioxydante la plus élevée a été mesurée dans les pépins. Par contre, il n'a pas été possible d'établir une différence claire entre les cépages.

Conclusion : Une grande variabilité a été trouvée parmi les divers cépages autochtones et non-autochtones étudiés par rapport à la teneur en polyphénols, ainsi que la composition en anthocyanes et l'activité antioxydante. Les pépins étaient la fraction de la baie de raisin à l'activité antioxydante la plus élevée suivie par les peaux et les pulpes, quel que soit le cépage.

Signification et impact de l'étude : Au Portugal, la plupart des vignobles possèdent des variétés portugaises de *Vitis vinifera* L. et d'autres espèces largement cultivées dans le monde. L'évaluation et la comparaison des composés phénoliques et de l'activité antioxydante de ces cépages n'ont pas été exploitées, à savoir sous les conditions environnementales des régions du *Douro* et du *Dão*. La variabilité observée dans le contenu phénolique des cépages étudiés confirme l'hypothèse que des facteurs génétiques jouent un rôle important dans la biosynthèse de ces composés et, par conséquent, sur l'activité antioxydante du raisin.

Mots clés : activité antioxydante, anthocyanes, cépage rouge, pulpe, pépin, pellicule

manuscript received 4th January 2013 - revised manuscript received 8th August 2013

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INTRODUCTION

Grapes are the chief dietary sources of anthocyanins (Rechkemmer and Pool-Zobel, 1996). Anthocyanins are mainly localized in berry skin (Jordão *et al*., 1998a), where they are found at concentrations of 200 to 5,000 mg/Kg of fresh grapes (Ribéreau-Gayon, 1982). Anthocyanin pigments are of noticeable importance in grapes and wines because of their dual role: first, they constitute an essential part of the sensory attributes, as their concentration, different forms and derivatives directly affect the color of the final wine and second, they are considered to have diverse biological properties and therefore are regarded as secondary metabolites with potential nutritional value. Grape anthocyanins are the 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin. Glucosylated derivatives of these anthocyanins, esterified at the C_6 position of glucose with acetyl or coumaroyl groups, have also been detected, yet generally at low concentrations. Malvidin is the predominant monomeric anthocyanin in grapes and is the reddest of all anthocyanins, providing the characteristic color of young red wines.

Anthocyanin concentration and profile is influenced by several factors such as varietal diversity (Kallithraka *et al*., 2005), soil type (Yokotsuka *et al*., 1999), environmental conditions (Jackson and Lombard, 1993; Mateus *et al*., 2002), vineyard management (Jordão *et al*., 1998b), and grape maturity (Jordão *et al*., 1998a). Additionally, some grape varieties can have a high anthocyanin concentration but a low extractability index (Romero-Cascales *et al*., 2005).

According to several studies, flavan-3-ols, flavonols and anthocyanins are the most important compounds that contribute to red grape and wine antioxidant proprieties (Beecher, 2003; Simonetti *et al*., 1997). For Rivero-Pérez *et al*. (2008), the free anthocyanin fraction is mainly responsible for the total antioxidant capacity and scavenger activity in red wines. However, other authors have reported that there is no correlation between antioxidant activity and spectral anthocyanin content in grapes and finished wines (Arnous *et al*., 2002; Kallithraka *et al*., 2005). For example, considering some individual anthocyanins (cyanidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside) from Italian wines, Di Majo *et al*. (2008) reported low correlations between these individual compounds and their antioxidant capacity (ranging from 0.29 to 0.54). In addition, Jordão *et al*. (2012) reported differences in the correlation values between individual anthocyanins and antioxidant activity during the maceration process. Thus, there has been conflicting evidence regarding the contribution of anthocyanins to red grape and wine antioxidant properties.

Dão and *Douro* are two important wine-producing regions in northeastern Portugal. The *Dão* region is sheltered on nearly all sides by high granite mountains and is situated in the transition zone between maritime and continental climate, with abundant rainfall in the winter months and long warm dry summers leading up to harvest. The *Douro* region, which is situated around the *Douro* River basin, is sheltered from Atlantic winds by several mountains and has a continental climate, with hot and dry summers and cold winters. Although Portuguese *Vitis vinifera* L. autochthonous cultivars are the most frequently used cultivars for the production of high quality red wines in both regions, other varieties from other countries (especially from France) have been introduced in the past few years.

A few studies have been performed on the anthocyanin profile of Portuguese grape varieties. However, these studies were restricted to a small number of varieties from southern (Dallas and Laureano, 1994a; Jordão *et al*., 1998a) and northern Portugal (Dallas and Laureano, 1994a; Dopico-García *et al*., 2008; Mateus *et al*., 2002) and did not address the antioxidant activity of the different grape berry fractions.

Thus, the purpose of this work was to evaluate the anthocyanin profile in grape skin as well the antioxidant activity in different grape berry fractions (skins, pulps and seeds) of 24 grape varieties from both Portuguese wine regions. The results obtained in this work could be useful for selecting winemaking techniques in order to improve anthocyanin extraction and hence increase the antioxidant capacity of the wines produced in these two regions. Finally, we should note that this is the first report of the anthocyanin content and antioxidant activity of grape cultivars from the *Dão* and *Douro* wine regions.

MATERIALS AND METHODS

1. Samples

In 2010, 24 grape varieties (*Vitis vinifera* L.) were harvested at technological maturity in good sanitary conditions from 6-year-old grapevines in two experimental vineyards of northeastern Portugal (one in the *Douro* region and the other in the *Dão* region). Grape berry samples (samples of 200 berries in duplicate) were picked randomly from 20 different vine plants in all possible conditions (e.g., different height and exposure to sunlight). All grape samples were kept frozen at -20 ºC until processing. The grape varieties studied are listed in Table 1.

Table 1. Grape variety and origin of the grape samples studied.

Grape variety	Autochthonous/ Non-autochthonous grape variety	Origin
Camarate	AG	Dão
Gewürztraminer*	NAG	Dão
Monvedro	AG	Dão
Moreto Boal	AG	Dão
Negro Mole	AG	Dão
Negro Mouro	AG	Dão
Alfrocheiro	AG	Douro
Alvarilhão	AG	Douro
Aramon	NAG	Douro
Bastardo	AG	Douro
Cabernet Franc	NAG	Douro
Carignan Noir	NAG	Douro
Cornifesto	AG	Douro
Gamay	NAG	Douro
Grenache	NAG	Douro
Jean	AG	Douro
Malvasia Preta	AG	Douro
Rufete	AG	Douro
Sousão	AG	Douro
Tinta Amarela	AG	Douro
Tinta Barca	AG	Douro
Tinta Barroca	AG	Douro
Tinta Miúda	AG	Douro
Tinto Cão	AG	Douro

AG - autochthonous grape variety; NAG - non-autochthonous grape variety; * white grape variety with colored skin

The evolutions of the climatic characteristics (rainfall, temperature and relative humidity) for the 2010 vintage in the two regions considered are summarized in Figure 1. In general, it was clear that during the three months (June, July and August) prior to sampling (at the end of September) the average temperature and average relative humidity was higher in the *Dão* region than in the *Douro* region. In addition, average rainfall for the months of July, August and September was lower in the *Dão* region.

2. General physico-chemical and phenolic parameters

Estimated alcohol degree, pH, titratable acidity and tartaric acid were measured directly from the must obtained after grape pressing, using the analytical methods recommended by the OIV (2006). Total phenolic compounds were determined by measuring absorbance at 280 nm (Ribéreau-Gayon *et al*., 1982). Non-flavonoids and flavonoids were determined using the method described by Kramling and Singleton (1969). Color intensity ($A_{420} + A_{520} + A_{620}$) and hue ($A_{420}/A_{520}$) were estimated using the method described by the OIV (2006). All analyses were done in duplicate.

3. Sample preparation for antioxidant activity and individual anthocyanin composition analysis

Skins, pulps and seeds were removed manually from the berries, washed separately several times with distilled water, and dried on blotting paper. Before the extraction process, seeds were crushed. The extraction process followed the procedure described by Sun *et al*. (1996): 50 mL of 80 % methanol followed by 50 mL of 75 % acetone, each for 3 h with agitation. After clarification of the suspension by centrifugation (10 min at 3500 rpm), all the extracts were combined. Aliquots of each extract were filtered (Whatman 0.45 µm) and frozen at -20 ºC until processing for antioxidant activity (skin, pulp and seed extracts) and individual anthocyanin composition (skin extracts) analysis. Extractions were done in duplicate for each grape berry fraction.

4. Antioxidant activity determination

The total antioxidant activity of each grape berry fraction was determined from the extracts according to two previously described methods: ABTS and DPPH. The ABTS method is based on the discoloration that occurs when the radical cation $ABTS^{\cdot+}$ is reduced to ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) (Re *et al*., 1999). The radical was generated by reaction of a 7 mM solution of ABTS in water with 2.45 mM potassium persulphate (1:1). The assay was made with 980 µL of $ABTS^{\cdot+}$ solution and 20 µL of sample (diluted 1:50 in water). The reaction was incubated for 15 min in darkness at room temperature, and then absorbance at 734 nm was measured.

The procedure using the DPPH method is described by Brand-Williams *et al.* (1995). Briefly, 0.1 mL of different sample concentrations was added to 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from the light. The reaction was carried out for 30 min at 20 ºC in closed Eppendorf tubes under shaking, and then absorbance at 515 nm was measured. Methanol was used as a blank reference. The antioxidant activity results were expressed as Trolox equivalents (TEAC mM), using the relevant calibration curve. All measurements were performed in duplicate.

5. Chromatographic analysis of individual anthocyanins

The analysis of individual anthocyanins from skin extracts was performed on a HPLC Dionex Ultimate 3000 Chromatographic System (Sunnyvale, California, USA) equipped with a quaternary pump

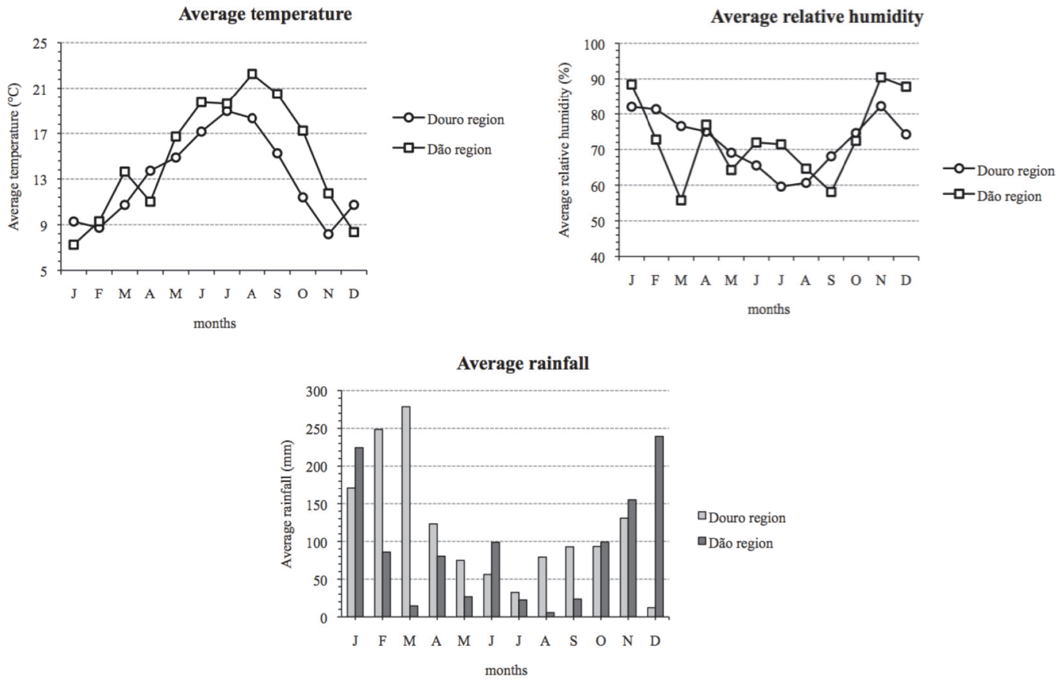

Figure 1. Evolution of climatic characteristics (temperature, relative humidity and rainfall) for the 2010 vintage in the two regions considered.

Model LPG-3400 A, an auto sampler Model ACC-3000, a thermostated column compartment (adjusted to 25 ºC) and a multiple Wavelength Detector MWD-300. The column (250 x 4.6 mm, particle size 5 µm) was a C_{18} Acclaim® 120 (Dionex, Sunnyvale, California, USA) protected by a guard column of the same material. The solvents were (A) 40 % formic acid, (B) pure acetonitrile and (C) bidistilled water. The anthocyanin composition was analyzed by HPLC using the method described by Dallas and Laureano (1994b). Thus, initial conditions were 25 % A, 10 % B and 65 % C, followed by a linear gradient from 10 to 30 % B and 65 to 45 % C for 40 min, with a flow rate of 0.7 mL/min. The injection volume was 40 µL. Detection was made at 520 nm using Chromeleon 6.8 software (Sunnyvale, California, USA). Individual anthocyanins were quantified by means of calibration curve obtained with standard solutions of malvidin-3-glucoside chloride (>95 % purity) from Extrasynthese (Genay, France). The chromatographic peaks of anthocyanins were identified according to reference data previously described by Dallas and Laureano (1994b). All analyses were done in duplicate.

6. Statistical analysis

The data are presented as mean ± standard deviation. To determine whether there is a statistically significant difference between the data obtained for antioxidant activity and for the diverse phenolic compounds quantified in the different grape varieties, an analysis of variance (ANOVA, one-way) and comparison of treatment means were carried out using Statistica 7 software (StatSoft, Tulsa, USA). Scheffle's test was applied to the data as comparison test to determine when samples are significantly different after ANOVA ($p < 0.05$). Principal component analysis (PCA) was used to analyze the data and study the relations between the grape varieties and their anthocyanin profile or antioxidant activity.

RESULTS AND DISCUSSION

1. General physico-chemical composition

The physico-chemical parameters of the grape varieties at technological maturity are summarized in Table 2. The estimated alcohol degree ranged from 9.53 % (v/v) (*Grenache*) to 14.84 % (v/v) (*Malvasia Preta*) with an average of 12.3 % (v/v). Titratable acidity, expressed as equivalent of tartaric acid, varied from 3.9 (*Moreto Boal*) to 13.5 g/L (*Jean* and *Tinta Miúda*) with an average of 7.3 g/L. The high titratable acidity and low pH values found in the majority of the grape varieties are probably a consequence of two main factors: their natural acidity and the ripening process (associated to low degradation rates of organic acids). In addition, these results are in agreement with the tartaric acid content of all grape varieties.

2. General phenolic composition and anthocyanin profile

It is well known that the genetic potential for polyphenol biosynthesis and the degree of ripeness of

Table 2. General physico-chemical composition of grape varieties at technological maturity.

Grape variety	Grape berry weight (g)^{a}	Must volume (mL)^{a}	Estimated alcohol degree (%, v:v)	pH	Titratable acidity (g/L tartaric acid)	Tartaric acid (g/L)
Camarate	241 ± 10^{a}	128 ± 5^{c}	11.00 ± 0.02^{e}	2.93 ± 0.04^{ef}	5.1 ± 0.1^{abc}	3.19 ± 0.23^{abc}
Gewürztraminer	444 ± 1^{l}	177 ± 1^{f}	12.76 ± 0.01^{m}	2.41± 0.00^{a}	8.8 ± 0.2^{fgh}	6.99 ± 0.02^{ijkl}
Monvedro	273 ± 8^{bcd}	130 ± 4^{cd}	12.35 ± 0.01^{k}	3.11± 0.00^{hij}	4.7 ± 0.3^{ab}	2.56 ± 0.02^{a}
Moreto Boal	325 ± 2^{fg}	152 ± 1^{e}	13.59 ± 0.02^{p}	3.17 ± 0.00^{ijk}	3.9 ± 0.3^{a}	3.35 ± 0.02^{bc}
Negro Mole	318 ± 8^{efg}	159 ± 4^{e}	11.47 ± 0.01^{f}	2.75 ± 0.00^{cd}	6.2 ± 0.1^{cd}	5.97 ± 0.08^{gh}
Negro Mouro	319 ± 10^{efg}	152 ± 5^{e}	12.15 ± 0.02^{j}	3.22 ± 0.00^{jk}	4.1 ± 0.1^{a}	3.38 ± 0.01^{bc}
Alfrocheiro	260 ± 8^{ab}	131± 6^{cd}	11.96 ± 0.02^{h}	2.91 ± 0.00^{ef}	6.0 ± 0.4^{bcd}	3.19 ± 0.23^{abc}
Alvarilhão	297 ± 9^{def}	144 ± 5de	13.45 ± 0.02^{o}	2.83 ± 0.00^{de}	8.0 ± 0.1^{ef}	6.95 ± 0.06^{ijkl}
Aramon	727 ± 6^{o}	322 ± 3^{k}	10.59 ± 0.02^{c}	2.72 ± 0.01^{bc}	9.5 ± 0.3^{gh}	7.70 ± 0.12^{k}
Bastardo	408 ± 2^{k}	181 ± 1^{f}	12.08 ± 0.02^{i}	3.49 ± 0.00^{m}	4.1 ± 0.2^{a}	2.49 ± 0.68^{a}
Cabernet Franc	268 ± 4^{abc}	66 ± 0^{a}	13.73 ± 0.01^{q}	2.94 ± 0.01^{ef}	6.0 ± 0.4^{bcd}	5.56 ± 0.04^{fg}
Carignan Noir	450 ± 3^{l}	179 ± 1^{f}	12.49 ± 0.02^{l}	3.14 ± 0.01^{hij}	8.5 ± 0.3^{fg}	6.01 ± 0.11^{gh}
Cornifesto	303 ± 6^{ef}	108 ± 2^{b}	14.01 ± 0.02^{r}	3.26 ± 0.00^{k}	6.0 ± 0.5^{bcd}	3.35 ± 0.02^{bc}
Gamay	487 ± 5^{m}	228 ± 2^{h}	11.67 ± 0.01^{g}	3.09 ± 0.09^{hi}	8.0 ± 0.1^{ef}	6.38 ± 0.01^{hij}
Grenache	378 ± 6^{ij}	203 ± 4^{g}	9.53 ± 0.01^{a}	3.22 ± 0.07^{jk}	10.0 ± 0.4^{h}	6.22 ± 0.00^{ghi}
Jean	387 ± 3^{jk}	179 ± 1^{f}	13.45 ± 0.02^{o}	2.92 ± 0.01^{ef}	13.5 ± 0.5^{i}	7.04 ± 0.05^{jkl}
Malvasia Preta	295 ± 8^{cde}	129 ± 4^{cd}	14.84 ±0.02^{s}	3.38 ± 0.01^{l}	8.5 ± 0.4^{fg}	3.88 ± 0.17^{cd}
Rufete	484 ± 8^{m}	269 ± 5^{j}	12.08 ± 0.01^{i}	3.05 ± 0.01^{gh}	5.5 ± 0.3^{bcd}	2.87 ± 0.02^{ab}
Sousão	336 ± 7^{gh}	178 ± 4^{f}	9.66 ± 0.02^{b}	2.62 ± 0.01^{b}	8.5 ± 0.1^{fg}	7.24 ± 0.02^{kl}
Tinta Amarela	530 ± 9^{n}	270 ± 9^{j}	10.93 ± 0.01^{d}	2.91 ± 0.02^{ef}	6.3 ± 0.1^{cd}	3.50 ± 0.16^{bc}
Tinta Barca	459 ± 9^{lm}	230 ± 5^{hi}	11.67 ± 0.02^{g}	2.88 ± 0.01^{ef}	9.0 ± 0.2^{fgh}	6.53 ± 0.03^{ijk}
Tinta Barroca	527 ± 7^{n}	246 ± 3^{i}	12.76 ± 0.01^{m}	3.15 ± 0.01^{hij}	5.5 ± 0.4^{bc}	4.57 ± 0.37^{de}
Tinta Miúda	358 ± 7^{hi}	197 ± 4^{g}	13.45 ± 0.02^{o}	2.88 ± 0.00^{ef}	13.5 ± 0.3^{i}	7.25 ± 0.24^{kl}
Tinto Cão	272 ± 6^{bcd}	111 ± 2^{b}	13.04 ± 0.01^{n}	2.94 ± 0.00^{fg}	6.8 ± 0.5^{de}	4.96 ± 0.16^{ef}

[a] Grape berry weight and must volume of 200 berries; data are the average of two replicates ± standard deviation; different letters above means indicate statistically significant differences between grape varieties ($p < 0.05$).

individual grape varieties may affect the polyphenolic content in grape berries at harvest. In addition, the concentration of phenolic compounds is highly influenced by viticulture and environmental factors such as sunlight, temperature, altitude, soil type, and water and nutritional status (Jackson and Lombard, 1993; Yokotsuka *et al*., 1999). As expected, due to the reasons mentioned above, differences in phenolic composition were observed among grape varieties (Table 3).

The total phenolic compounds, expressed as equivalent of gallic acid, ranged from 989 to 3033 mg/L with an average of 1608.5 mg/L. The highest concentration of total phenols (ranging from 2119 to 3033 mg/L) was detected in *Tinta Barca*, *Tinta Barroca*, *Sousão* and *Tinta Miúda*, while *Camarate*, *Moreto Boal*, *Cornifesto* and *Grenache* showed the lowest values (ranging from 989 to 1088 mg/L).

The interest of winemakers for grape polyphenolic content is increasing, as it offers tools to influence the color, bitterness, astringency, 'mouth-feel' and 'age-ability' of wines. Therefore, the color of red wines and its evolution depend not only on the winemaking and aging processes, but also on the potential concentration of anthocyanins in grape skin as well as the easiness of anthocyanin extraction from skin into must (Ortega-Regules *et al*., 2006).

The individual anthocyanin composition of the skin extracts of the grape varieties at technological maturity is presented in Table 4. Malvidin-3-glucoside was the major individual anthocyanin (concentrations ranging from 0.62 to 6.09 mg/g of skin) in all varieties except *Alvarilhão* and *Rufete*, where the major individual anthocyanins were peonidin-3-glucoside (1.04 mg/g of skin) and malvidin-3-*p*-coumaroyl glucoside (1.48 mg/g of skin), respectively. Malvidin-3-*p*-coumaroyl glucoside was the second main individual anthocyanin for the majority of the varieties and the values ranged from 0.12 to 3.44 mg/g of skin. These results are in agreement with previous findings in other grape varieties (Dallas and Laureano, 1994a; Jordão *et al*., 1998a; Kallithraka *et al*., 2005; Ortega-Regules *et al*., 2006). According to Ribéreau-Gayon *et al*. (2006), malvidin derivative forms are stable molecules and their presence give stability to the wine during the winemaking process because of their relative resistance to oxidation. Mateus *et al*.

(2002) found that malvidin-3-glucoside and its acylated esters were the major anthocyanin monoglucosides in *Touriga Nacional* and *Touriga Francesa* at harvest date.

In general, cyanidin derivatives (cyanidin-3-acetyl glucoside and petunidin-3-acetyl glucoside) were the less abundant individual anthocyanins (ranging from 0.01 to 0.05 and 0.01 to 0.39 mg/g of skin, respectively), which is in line with the results published by several authors (Dimitrovska *et al*., 2011; Ortega-Meder *et al*., 1994; Roggero *et al*., 1986). In addition, these two anthocyanins were not detected in a great number of the grape varieties studied. According to Di Stefano and Flamini (2008), cyanidin is the precursor of peonidin-3-glucoside by the action of the UFGT (which transform cyanidin into cyanidin-3-glucoside) and MT enzymes (which transform cyanidin-3-glucoside into peonidin-3-glucoside).

The glucoside group was the main anthocyanin chemical group in all grape varieties, ranging from 1.40 (*Rufete*) to 7.09 (*Carignan Noir*) mg/g of skin (sum of all five 3-glucoside anthocyanins). The coumaroyl glucoside group was the second most important group, ranging from 0.23 (*Bastardo*) to 2.87 (*Tinto Cão*) mg/g of skin, except in four varieties (*Camarate*, *Gewürztraminer*, *Monvedro* and *Jean*) where the acetyl glucoside group was the second main group. A high concentration of *p*-coumaroyl-anthocyanin derivatives, mainly from malvidin and petunidin, has been associated with warm climates (Downey *et al*., 2006). However, according to Férnandez-Lopez *et al*. (1998) it is commonly accepted that the anthocyanin composition of each cultivar is closely linked to its genetic inheritance and, from a qualitative point of view, is quite independent of seasonal conditions or production area.

The acetyl glucoside group, which participates in an intra-molecular copigmentation process leading to an increase in wine color intensity (Ortega-Regules *et al*., 2006), was the minor group, ranging from 0.01 (*Grenache*) to 2.03 (*Gewürztraminer*) mg/g of skin. Although the anthocyanin profile may be complex and quite different for each variety studied, in general *Carignan Noir*, *Monvedro* and *Negro Mole* showed the highest individual anthocyanin concentration while *Bastardo*, *Aramon*, *Grenache* and *Alvarilhão* showed the lowest concentration. It is important to note that grape anthocyanin concentration is largely

Table 3. General phenolic composition of grape varieties at technological maturity.

Grape variety	Total phenolic compounds (mg/L gallic acid)	Flavonoid compounds (mg/L gallic acid)	Non-flavonoid compounds (mg/L gallic acid)	Color intensity (abs.u.)[a]	Color hue (abs.u.)[a]
Camarate	989 ± 13[a]	946 ± 11[abc]	43 ± 2[a]	13.57 ± 0.83[h]	6.31 ± 0.35[cde]
Gewürztraminer	1124 ± 17[ab]	1031 ± 16[bcd]	93 ± 1[abc]	9.34 ± 0.24[cde]	7.96 ± 0.25[ghi]
Monvedro	1240 ± 14[abc]	1118 ± 13[bcde]	122 ± 1[bcd]	7.62 ± 0.16[b]	7.46 ± 0.15[efgh]
Moreto Boal	1032 ± 39[a]	961 ± 9[abc]	71 ± 3[ab]	5.21 ± 0.13[a]	11.33 ± 0.26[j]
Negro Mole	1375 ± 17[abcd]	1278 ± 16[defg]	97 ± 1[abc]	10.15 ± 0.38[ef]	5.54 ± 0.08[abc]
Negro Mouro	1593 ± 26[def]	1408 ± 23[efgh]	185 ± 3[def]	12.17 ± 0.11[gh]	6.46 ± 0.12[cdef]
Alfrocheiro	1492 ± 13[bcd]	1262 ± 11[defg]	229 ± 2[efgh]	9.94 ± 0.20[def]	7.79 ± 0.11[fghi]
Alvarilhão	1820 ± 39[fgh]	1582 ± 34[ghi]	238 ± 5[efgh]	9.17 ± 0.16[cde]	7.28 ± 0.22[defgh]
Aramon	1590 ± 13[def]	1341 ± 11[fgh]	248 ± 2[fghi]	8.56 ± 0.14[bcd]	8.74 ± 0.10[i]
Bastardo	2024 ± 37[gh]	1662 ± 30[hi]	362 ± 7[k]	9.93 ± 0.23[def]	8.59 ± 0.25[hi]
Cabernet Franc	1900 ± 17[fgh]	1535 ± 14[fghi]	365 ± 6[k]	13.26 ± 0.15[h]	8.26 ± 0.07[hi]
Carignan Noir	1476 ± 22[bcd]	1268 ± 19[defg]	208 ± 5[efg]	10.28 ± 0.87[ef]	8.35 ± 0.51[hi]
Cornifesto	1088 ± 18[ab]	874 ± 13[a]	214 ± 3[efg]	8.25 ± 0.04[bc]	6.82 ± 0.10[cdefg]
Gamay	1529 ± 18[cde]	1309 ± 12[efgh]	220 ± 2[efgh]	12.86 ± 0.43[h]	8.08 ± 0.48[ghi]
Grenache	1038 ± 52[a]	907 ± 9[ab]	131 ± 7[bcd]	5.41 ± 0.60[a]	7.43 ± 0.35[efgh]
Jean	1530 ± 24[cde]	1334 ± 10[efgh]	196 ± 5[defg]	10.99 ± 0.30[fg]	5.94 ± 0.29[bcd]
Malvasia Preta	1352 ± 28[abcd]	1083 ± 23[bcde]	269 ± 6[ghij]	10.05 ± 0.14[ef]	6.18 ± 0.27[bcde]
Rufete	1297 ± 33[abc]	1133 ± 28[bcde]	164 ± 4[cde]	11.16 ± 0.33[fg]	5.86 ± 0.00[bc]
Sousão	2130 ± 74[h]	1898 ± 12[i]	232 ± 8[efgh]	18.68 ± 0.21[jk]	5.78 ± 0.22[bc]
Tinta Amarela	1490 ± 28[bcd]	1197 ± 23[cdef]	294 ± 6[hijk]	16.22 ± 0.37[i]	4.86 ± 0.03[ab]
Tinta Barca	3033 ± 19[i]	2700 ± 16[j]	333 ± 13[jk]	19.15 ± 0.12[k]	5.99 ± 0.02[bcd]
Tinta Barroca	2783 ± 13[i]	2418 ± 18[j]	365 ± 15[k]	17.68 ± 0.10[f]	6.14 ± 0.16[bcde]
Tinta Miúda	2119 ± 37[h]	1857 ± 32[i]	263 ± 5[ghij]	21.68 ± 0.16[l]	4.19 ± 0.12[a]
Tinto Cão	1562 ± 33[def]	1254 ± 26[defg]	308 ± 11[ijk]	13.35 ± 0.42[h]	5.78 ± 0.18[bc]

[a] abs.u. – absorbance units referred to a 1 mm path length cell used; data are the average of two replicates ± standard deviation; different letters above means indicate statistically significant differences between grape varieties ($p < 0.05$).

due, among other factors, to the berry size of each grape variety. On the other hand, *Gewürztraminer*, *Monvedro*, *Moreto Boal*, *Negro Mole*, *Alvarilhão*, *Cabernet Franc*, *Jean* and *Tinta Amarela* presented the widest variety of individual anthocyanins while *Rufete* presented the narrowest variety of individual anthocyanins quantified.

To better understand the relationship between grape variety and anthocyanin concentration, a principal component analysis (PCA) was performed (Figure 2A). The first two principal components (PCs) explained 92.22 % of the total variance and showed that grape varieties can be distinguished according to their individual anthocyanin concentration. Figure 2A shows the corresponding loading plots that established the relative importance of each variable. The first principal component (PC1), which explained 81.89 % of the variance, was negatively correlated with the variables malvidin-3-glucoside and malvidin-3-*p*-coumaroyl glucoside. The second PC (PC2, 10.33 % of the variance) was positively correlated with the variable peonidin-3-glucoside and negatively correlated with malvidin-3-acetyl glucoside. Five groups could be distinguished. The varieties *Camarate*, *Gewürztraminer*, *Monvedro*, *Negro Mole*, *Negro Mouro*, *Cabernet Franc*, *Carignan Noir*, *Cornifesto* and *Tinta Barroca* were rather grouped on the negative side of PC1, due to their high content in malvidin-3-glucoside (ranging from 4.10 to 6.09 mg/g of skin) and malvidin-3-*p*-coumaroyl glucoside (ranging from 0.88 to 3.44 mg/g of skin). The varieties *Moreto Boal*, *Monvedro*, *Gewürztraminer* and *Camarate* appeared in the positive part of PC2, due to their high content in malvidin-3-acetyl glucoside (ranging from 1.01 to 1.65 mg/g of skin).

From the description presented above, anthocyanins could be considered useful markers to distinguish grape varieties; however, this characteristic should be used with caution since anthocyanin concentration is influenced not only by genetic background but also by agroecological factors, such as maturation (Conde *et al*., 2007; Jordão *et al*., 1998a), ripening stage (Segade *et al*., 2008), climate (Gil and Yuste, 2004), stress levels (Gatto *et al*., 2008) and cultural practices (Jordão *et al*., 1998b).

3. Antioxidant activity from different grape berry fractions

The data in Table 5 show the antioxidant activity quantified in the different grape berry fractions (pulps, skins and seeds) from the grape varieties studied. The highest antioxidant activity was found in seeds (ranging from 77.59 to 867.81 and from 75.52 to 363.47 µmol/g of seed, for ABTS and DPPH method, respectively), followed by skins (ranging from 1.13 to 292.05 and from 1.78 to 299.99 µmol/g of skin, for ABTS and DPPH method, respectively) and pulps

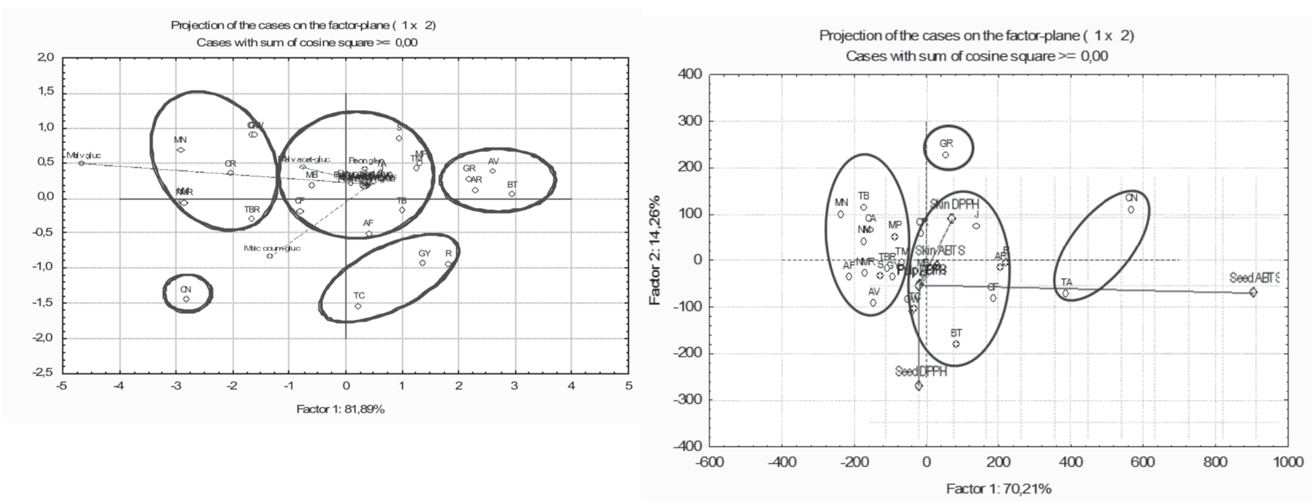

Figure 2. Principal component analysis score plot (PC1 and PC2) of grape varieties: individual anthocyanins (A) and antioxidant activity (B).

CA, Camarate; GW, Gewürztraminer; MN, Monvedro; MB, Moreto Boal; NM, Negro Mole; NMR, Negro Mouro; AF, Alfrocheiro; AV, Alvarilhão; AR, Aramon; BT, Bastardo; CF, Cabernet Franc; CN, Carignan Noir; CR, Cornifesto; GY, Gamay; GR, Grenache; J, Jean; MP, Malvasia Preta; R, Rufete; S, Sousão; TA, Tinta Amarela; TB, Tinta Barca; TBR, Tinta Barroca; TM, Tinta Miúda; TC, Tinto Cão.

Delp gluc, delphinidin-3-glucoside; Cyan gluc, cyanidin-3-glucoside; Petun gluc, petunidin-3-glucoside; Peon gluc, peonidin-3-glucoside; Malv gluc, malvidin-3-glucoside; Cyan acet-gluc, cyanidin-3 acetyl glucoside; Petun acet-gluc, petunidin-3-acetyl glucoside; Peon acet-gluc, peonidin-3-acetyl glucoside; Malv acet-gluc, malvidin-3-acetyl glucoside; Peon coum-gluc, peonidin-

Table 4. Individual anthocyanins quantified in grape skin at technological maturity.

Grape variety	Delp gluc	Cyan gluc	Petun gluc	Peon gluc	Malv gluc	Cyan acet-gluc	Petun acet-gluc	Peon acet-gluc	Malv acet-gluc	Petun coum-gluc	Peon coum-gluc	Malv coum-gluc
Camarate	n.d.	0.05 ± 0.01^{e}	0.22 ± 0.01^{defg}	0.49 ± 0.02^{e}	5.08 ± 0.02^{g}	0.02 ± 0.0^{bc}	0.02 ± 0.00^{a}	0.12 ± 0.01^{g}	1.29 ± 0.07^{i}	0.12 ± 0.00^{g}	0.11 ± 0.00^{def}	0.90 ± 0.17^{def}
Gewürztraminer	0.21 ± 0.05^{cd}	0.01 ± 0.00^{a}	0.44 ± 0.11^{j}	0.26 ± 0.02^{cd}	4.92 ± 0.04^{g}	0.03 ± 0.00^{d}	0.39 ± 0.00^{d}	0.07 ± 0.00^{ef}	1.54 ± 0.03^{jk}	0.39 ± 0.00^{k}	0.07 ± 0.00^{bcde}	0.88 ± 0.13^{def}
Monvedro	0.60 ± 0.00^{f}	0.01 ± 0.01^{a}	0.94 ± 0.02^{m}	0.30 ± 0.05^{d}	6.09 ± 0.04^{i}	0.05 ± 0.00^{e}	0.14 ± 0.00^{bc}	0.01 ± 0.00^{a}	1.65 ± 0.01^{k}	0.11 ± 0.01^{g}	0.09 ± 0.00^{cdef}	1.48 ± 0.03^{efg}
Moreto Boal	0.08 ± 0.02^{ab}	0.01 ± 0.00^{a}	0.06 ± 0.00^{ab}	0.49 ± 0.03^{e}	3.96 ± 0.08^{f}	0.01 ± 0.00^{ab}	0.06 ± 0.00^{a}	0.11 ± 0.00^{g}	1.01 ± 0.00^{h}	0.10 ± 0.00^{fg}	0.12 ± 0.01^{ef}	1.27 ± 0.03^{efg}
Negro Mole	0.04 ± 0.01^{ab}	0.01 ± 0.00^{a}	0.24 ± 0.05^{efgh}	0.51 ± 0.10^{e}	5.92 ± 0.00^{hi}	0.02 ± 0.00^{bc}	0.21 ± 0.00^{c}	0.11 ± 0.00^{g}	1.43 ± 0.07^{j}	0.08 ± 0.00^{ef}	0.11 ± 0.01^{def}	2.21 ± 0.07^{gh}
Negro Mouro	0.03 ± 0.01^{ab}	n.d.	0.21 ± 0.00^{cdefg}	0.54 ± 0.05^{ef}	5.88 ± 0.04^{hi}	0.02 ± 0.00^{bc}	0.08 ± 0.01^{ab}	0.10 ± 0.00^{fg}	1.45 ± 0.04^{j}	0.03 ± 0.01^{a}	0.09 ± 0.03^{cdef}	2.23 ± 0.05^{gh}
Alfrocheiro	0.03 ± 0.01^{ab}	n.d.	0.16 ± 0.01^{bcdef}	0.14 ± 0.00^{b}	2.90 ± 0.15^{e}	n.d.	n.d.	0.04 ± 0.01^{abcd}	0.18 ± 0.01^{bcde}	0.06 ± 0.00^{cde}	0.04 ± 0.01^{ab}	1.46 ± 0.15^{efg}
Alvarilhão	0.08 ± 0.01^{ab}	0.24 ± 0.01^{h}	0.12 ± 0.00^{bcd}	1.04 ± 0.04^{h}	0.99 ± 0.05^{ab}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.06 ± 0.00^{cde}	0.05 ± 0.00^{ab}	0.01 ± 0.00^{a}	0.12 ± 0.01^{ef}	0.12 ± 0.03^{a}
Aramon	0.05 ± 0.00^{ab}	0.03 ± 0.00^{bc}	0.08 ± 0.00^{ab}	0.17 ± 0.01^{bc}	1.24 ± 0.01^{b}	n.d.	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.15 ± 0.01^{bcde}	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}	0.32 ± 0.08^{bc}
Bastardo	0.02 ± 0.00^{a}	0.03 ± 0.00^{bc}	0.01 ± 0.00^{a}	0.13 ± 0.00^{ab}	0.62 ± 0.00^{a}	n.d.	n.d.	0.02 ± 0.00^{abc}	0.05 ± 0.00^{ab}	0.02 ± 0.00^{a}	0.05 ± 0.00^{abc}	0.16 ± 0.01^{a}
Cabernet Franc	0.95 ± 0.00^{g}	0.04 ± 0.00^{de}	1.03 ± 0.01^{m}	0.17 ± 0.02^{bc}	4.10 ± 0.13^{f}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.20 ± 0.01^{i}	0.27 ± 0.00^{ef}	0.22 ± 0.01^{i}	0.02 ± 0.00^{a}	1.52 ± 0.10^{fgh}
Carignan Noir	0.34 ± 0.05^{e}	0.02 ± 0.01^{ab}	0.57 ± 0.01^{k}	0.56 ± 0.03^{ef}	5.60 ± 0.37^{h}	n.d.	n.d.	0.04 ± 0.01^{abcd}	0.53 ± 0.01^{g}	0.16 ± 0.01^{h}	0.35 ± 0.03^{g}	3.44 ± 0.43^{i}
Cornifesto	0.03 ± 0.01^{a}	n.d.	0.10 ± 0.00^{abc}	0.56 ± 0.03^{ef}	5.60 ± 0.37^{h}	n.d.	n.d.	0.02 ± 0.00^{abc}	0.17 ± 0.00^{bcde}	0.06 ± 0.00^{cde}	0.03 ± 0.00^{a}	1.38 ± 0.13^{efg}
Gamay	0.02 ± 0.01^{a}	n.d.	0.08 ± 0.02^{ab}	0.12 ± 0.00^{ab}	1.77 ± 0.03^{c}	n.d.	n.d.	0.04 ± 0.00^{abcd}	0.23 ± 0.00^{cde}	0.02 ± 0.00^{a}	0.11 ± 0.02^{def}	1.61 ± 0.01^{fgh}
Grenache	0.25 ± 0.00^{cd}	0.04 ± 0.00^{de}	0.14 ± 0.01^{bcde}	0.18 ± 0.00^{bcd}	1.44 ± 0.01^{bc}	n.d.	n.d.	n.d.	0.01 ± 0.00^{a}	0.05 ± 0.00^{bcd}	0.05 ± 0.00^{abc}	0.17 ± 0.01^{a}
Jean	0.09 ± 0.01^{b}	0.02 ± 0.00^{ab}	0.20 ± 0.01^{cdef}	0.27 ± 0.01^{cd}	2.75 ± 0.05^{de}	0.02 ± 0.00^{bc}	0.04 ± 0.00^{a}	0.03 ± 0.03^{abc}	0.89 ± 0.07^{h}	0.04 ± 0.01^{abc}	0.02 ± 0.00^{a}	0.77 ± 0.02^{cde}
Malvasia Preta	0.21 ± 0.00^{cd}	0.06 ± 0.01^{ef}	0.31 ± 0.00^{ghi}	0.55 ± 0.02^{ef}	2.32 ± 0.06^{d}	n.d.	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.10 ± 0.00^{abc}	0.02 ± 0.00^{a}	0.04 ± 0.02^{abc}	0.28 ± 0.05^{ab}
Rufete	n.d.	n.d.	n.d.	0.01 ± 0.00^{a}	1.39 ± 0.01^{bc}	n.d.	n.d.	0.02 ± 0.00^{ab}	0.14 ± 0.01^{bcde}	0.03 ± 0.00^{a}	0.05 ± 0.00^{abc}	1.48 ± 0.03^{efg}
Sousão	0.21 ± 0.01^{cd}	0.09 ± 0.01^{g}	0.33 ± 0.01^{hi}	1.34 ± 0.01^{i}	2.76 ± 0.05^{de}	n.d.	n.d.	n.d.	0.06 ± 0.00^{ab}	0.02 ± 0.00^{a}	0.04 ± 0.00^{abc}	0.18 ± 0.08^{a}
Tinta Amarela	0.58 ± 0.02^{f}	0.07 ± 0.01^{fg}	0.74 ± 0.01^{l}	0.69 ± 0.02^{g}	2.91 ± 0.07^{e}	0.02 ± 0.00^{bc}	0.04 ± 0.01^{a}	0.03 ± 0.01^{abcd}	0.10 ± 0.01^{ab}	0.05 ± 0.00^{bcd}	0.06 ± 0.01^{abcd}	0.65 ± 0.01^{cde}
Tinta Barca	0.34 ± 0.00^{e}	0.04 ± 0.00^{de}	0.46 ± 0.01^{jk}	0.23 ± 0.00^{bcd}	2.41 ± 0.01^{d}	n.d.	n.d.	0.05 ± 0.00^{bcde}	0.13 ± 0.01^{bcd}	0.04 ± 0.01^{abc}	n.d.	0.98 ± 0.01^{def}
Tinta Barroca	0.21 ± 0.01^{cd}	0.06 ± 0.00^{ef}	0.41 ± 0.01^{ij}	0.57 ± 0.00^{efg}	4.93 ± 0.05^{g}	n.d.	n.d.	0.07 ± 0.00^{de}	0.26 ± 0.00^{def}	0.11 ± 0.01^{g}	0.13 ± 0.01^{f}	1.94 ± 0.03^{fgh}
Tinta Miúda	0.20 ± 0.01^{c}	0.01 ± 0.00^{a}	0.25 ± 0.01^{fgh}	0.64 ± 0.03^{fg}	2.31 ± 0.06^{d}	n.d.	0.02 ± 0.00^{a}	0.04 ± 0.00^{bcde}	0.26 ± 0.00^{def}	0.02 ± 0.01^{a}	0.11 ± 0.02^{def}	0.41 ± 0.04^{bcd}
Tinto Cão	0.27 ± 0.01^{d}	0.01 ± 0.00^{a}	0.39 ± 0.01^{ij}	0.13 ± 0.02^{ab}	2.65 ± 0.01^{de}	n.d.	0.01 ± 0.00^{a}	0.16 ± 0.01^{h}	0.37 ± 0.00^{f}	0.25 ± 0.01^{j}	0.05 ± 0.03^{abc}	2.57 ± 0.02^{hi}

Delp gluc, delphinidin-3-glucoside; Cyan gluc, cyanidin-3-glucoside; Petun gluc, petunidin-3-glucoside; Peon gluc, peonidin-3-glucoside; Malv gluc, malvidin-3-glucoside; Cyan acet-gluc, cyanidin-3- acetyl glucoside; Petun acet-gluc, petunidin-3-acetyl glucoside; Peon acet-gluc, peonidin-3-acetyl glucoside; Malv acet-gluc, malvidin-3-acetyl glucoside; Peon coum-gluc, peonidin-3-*p*-coumaroyl glucoside; Malv coum-gluc, malvidin-3-*p*-coumaroyl glucoside; individual anthocyanins are expressed as malvidin-3-glucoside equivalents (mg/g of skin); n.d., not detected; data are the average of two replicates ± standard deviation; different letters above means indicate statistically significant differences between grape varieties ($p < 0.05$).

Table 5. Antioxidant activities of grape skin, pulp and seed extracts as measured by the ABTS and DPPH methods in grape varieties at technological maturity.

Grape variety	Skins		Pulps		Seeds	
	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH
Camarate	47.64 ± 0.62^{abc}	58.14 ± 2.02^{efgh}	2.57 ± 0.00^{kl}	3.13 ± 0.27^{j}	161.13 ± 7.23^{bcde}	129.00 ± 18.91^{abc}
Gewürztraminer	78.78 ± 7.45^{gh}	45.30 ± 0.00^{defg}	2.14 ± 0.07^{ijk}	2.02 ± 0.05^{ef}	276.21 ± 6.03^{fghi}	330.89 ± 1.89^{ij}
Monvedro	34.48 ± 0.62^{b}	66.71 ± 0.00^{ghi}	1.58 ± 0.02^{fghi}	1.84 ± 0.07^{de}	77.59 ± 0.00^{a}	96.91 ± 11.35^{ab}
Moreto Boal	93.28 ± 1.86^{h}	9.63 ± 1.09^{ab}	3.65 ± 0.02^{n}	3.12 ± 0.03^{j}	310.31 ± 1.21^{hi}	210.56 ± 9.45^{def}
Negro Mole	71.78 ± 4.96^{fg}	88.11 ± 2.02^{hi}	0.95 ± 0.04^{bcde}	1.05 ± 0.09^{b}	137.26 ± 4.82^{bcd}	185.15 ± 15.13^{cde}
Negro Mouro	57.74 ± 4.96^{cdef}	48.60 ± 3.78^{defg}	1.95 ± 0.07^{ijk}	2.48 ± 0.01^{ghi}	145.79 ± 14.47^{bcd}	239.97 ± 39.71^{efg}
Alfrocheiro	47.20 ± 3.72^{abc}	15.34 ± 4.04^{bc}	0.04 ± 0.03^{a}	0.18 ± 0.06^{a}	105.72 ± 1.21^{ab}	229.27 ± 54.83^{def}
Alvarilhão	9.03 ± 3.10^{a}	38.79 ± 20.18^{defg}	0.05 ± 0.03^{a}	2.01 ± 0.06^{ef}	174.77 ± 4.82^{bcde}	300.14 ± 3.78^{ghij}
Aramon	133.21 ± 4.96^{i}	203.27 ± 16.40^{k}	4.80 ± 0.22^{o}	2.59 ± 0.07^{hi}	505.52 ± 16.88^{l}	314.84 ± 1.89^{hij}
Bastardo	1.13 ± 0.62^{a}	1.78 ± 0.01^{a}	0.82 ± 0.07^{bcd}	2.02 ± 0.05^{ef}	410.05 ± 19.29^{jk}	363.47 ± 11.82^{j}
Cabernet Franc	52.91 ± 1.86^{bcdef}	30.32 ± 1.01^{cde}	1.71 ± 0.12^{fghi}	1.48 ± 0.02^{cd}	506.37 ± 10.85^{l}	264.87 ± 4.73^{fghi}
Carignan Noir	192.01 ± 9.93^{j}	182.37 ± 1.26^{k}	3.42 ± 0.13^{nm}	2.25 ± 0.01^{fgh}	867.81 ± 18.08^{n}	130.34 ± 9.45^{abc}
Cornifesto	52.91 ± 0.62^{bcdef}	23.40 ± 3.47^{bcd}	1.16 ± 0.07^{cdef}	1.25 ± 0.08^{bc}	306.89 ± 44.61^{hi}	106.27 ± 5.67^{ab}
Gamay	42.38 ± 3.10^{bc}	68.22 ± 11.35^{ghi}	1.92 ± 0.07^{hij}	2.24 ± 0.02^{fgh}	224.21 ± 19.29^{efgh}	253.57 ± 19.24^{efgh}
Grenache	33.16 ± 6.21^{b}	299.99 ± 5.04^{l}	2.95 ± 0.06^{lm}	2.71 ± 0.05^{i}	348.67 ± 14.47^{ij}	75.52 ± 11.35^{a}
Jean	63.88 ± 13.65^{defg}	94.08 ± 12.61^{i}	3.30 ± 0.56^{nm}	1.12 ± 0.06^{bc}	453.52 ± 20.49^{kl}	122.31 ± 1.89^{abc}
Malvasia Preta	1.57 ± 1.24^{a}	3.21 ± 1.01^{a}	0.55 ± 0.04^{ab}	1.12 ± 0.06^{bc}	237.85 ± 16.88^{efgh}	95.57 ± 13.24^{ab}
Rufete	37.55 ± 2.48^{bc}	63.76 ± 7.57^{fghi}	1.86 ± 0.03^{ghij}	1.09 ± 0.05^{b}	539.62 ± 69.92^{l}	189.49 ± 13.24^{cde}
Sousão	68.27 ± 0.00^{efg}	43.88 ± 4.04^{defg}	1.30 ± 0.16^{fgh}	1.09 ± 0.02^{b}	189.26 ± 1.21^{cdef}	249.65 ± 5.67^{efg}
Tinta Amarela	45.89 ± 1.86^{bcd}	7.75 ± 0.96^{ab}	0.63 ± 0.28^{abc}	0.49 ± 0.00^{a}	711.81 ± 14.47^{m}	219.92 ± 0.00^{def}
Tinta Barca	50.28 ± 0.62^{bcde}	137.33 ± 7.06^{j}	0.16 ± 0.06^{a}	0.49 ± 0.01^{a}	122.77 ± 3.62^{abc}	161.09 ± 11.35^{bcd}
Tinta Barroca	7.20 ± 1.96^{a}	87.39 ± 1.01^{hi}	1.24 ± 0.13^{defg}	1.12 ± 0.00^{bc}	206.31 ± 13.26^{defg}	237.30 ± 1.89^{efg}
Tinta Miúda	292.05 ± 3.72^{k}	32.24 ± 6.73^{cdef}	2.55 ± 0.06^{kl}	2.14 ± 0.11^{efg}	236.99 ± 22.91^{efgh}	239.97 ± 1.89^{efg}
Tinto Cão	123.55 ± 7.45^{i}	25.33 ± 4.04^{bcd}	2.40 ± 0.15^{jkl}	2.48 ± 0.01^{ghi}	283.88 ± 31.34^{ghi}	318.35 ± 14.18^{hij}

Data are the average of two replicates ± standard deviation; values are expressed as µmol/g of skin, pulp or seed; different letters above means indicate statistically significant differences between grape varieties ($p < 0.05$).

(ranging from 0.04 to 4.80 and from 0.18 to 3.13 µmol/g of pulp, for ABTS and DPPH method, respectively). In addition, the average values were 314.17, 68.24 and 1.82 µmol/g, respectively for seeds, skins and pulps considering the ABTS method and 211.01, 89.78 and 1.72 µmol/g, respectively for seeds, skins and pulps considering the DPPH method. One possible explanation for this distribution in the different grape berry fractions could be the higher amount of polyphenols such as monomeric flavanols (catechin and epicatechin), dimeric, trimeric and polymeric proanthocyanidins, and phenolic acids in seeds compared to skins (Di Majo *et al*., 2008; Yilmaz and Toledo, 2004). These findings are in agreement with previous studies conducted with other red *Vitis vinifera* grape varieties (Poudel *et al*., 2008; Xu *et al*., 2010).

Taking into account the antioxidant activity of the red grape varieties analyzed here, a large variation was found in the different grape berry fractions (Table 5). For seeds and considering the ABTS method, *Carignan Noir*, *Tinta Amarela*, *Rufete*, *Cabernet Franc*, *Aramon*, *Jean* and *Bastardo* showed the highest antioxidant activity values (ranging from 410.05 to 867.81 µmol/g of seed; $p < 0.05$) while *Monvedro* and *Alfrocheiro* showed the lowest antioxidant values (77.59 and 105.72 µmol/g of seed, respectively; $p < 0.05$). However, considering the DPPH method, *Bastardo*, *Gewürztraminer*, *Tinto Cão*, *Aramon* and *Alvarilhão* showed the highest antioxidant activity values (ranging from 300.14 to 363.47 µmol/g of seed; $p < 0.05$) while *Monvedro*, *Grenache* and *Malvasia Preta* showed the lowest antioxidant values (ranging from 75.52 to 96.91 µmol/g of seed; $p < 0.05$).

For skins and considering the ABTS method, *Tinta Miúda*, *Carignan Noir*, *Aramon* and *Tinto Cão* showed the highest antioxidant activity values (ranging from 123.55 to 292.05 µmol/g of skin; $p < 0.05$) while *Bastardo*, *Malvasia Preta* and *Tinta Barroca* showed the lowest antioxidant values (ranging from 1.13 to 7.20 µmol/g of skin, respectively; $p < 0.05$). The results obtained by the DPPH method showed similar distribution of high (*Carignan Noir* and *Aramon*) and low (*Malvasia Preta* and *Bastardo*) antioxidant activity values. Finally, for pulps, high antioxidant activity values were found for *Aramon*, *Moreto Boal* and *Carignan Noir* (4.80, 3.65 and 3.42 µmol/g of pulp for ABTS method, respectively) while low antioxidant values were found for *Alfrocheiro*, *Alvarilhão*, *Tinta Barca* and *Malvasia Preta* (0.04, 0.05, 0.16 and 0.55 µmol/g of pulp for ABTS method, respectively). Overall, there is no clear difference between the autochthonous and non-autochthonous grape varieties and the grape samples from the two wine regions considered. From these results, it is clear that the antioxidant activity values depend on the method used. According to Villaño *et al*. (2006), this divergence is due to the different reagents of the polyphenols with each method applied. For Wang *et al*. (2004), $ABTS^{\cdot+}$ and DPPH radicals have a different stereochemical structure and a different method of genesis and thus they lend, after the reaction with the antioxidants, a qualitatively different response to the inactivation of their radical. Some authors reported that no single assay can provide all the information needed to evaluate antioxidant capacity, and multiple assays are therefore required to build up an antioxidant profile of particular foodstuffs (Rivero-Pérez *et al*., 2007).

To highlight the relation between grape varieties and their antioxidant activity in the different grape berry fractions (pulps, skins and seeds), a PCA was applied (Figure 2B). The PCA of the antioxidant activity values obtained by two methods showed that the first two PCs explained 84.47 % of the total variance. The first PC (70.21 % of the variance) was positively correlated with seeds ABTS and the second PC (14.26 % of the variance) was positively correlated with skins DPPH and negatively correlated with seeds DPPH. Grape varieties appeared grouped due to their different antioxidant activity of skins, seeds and pulps. The grape varieties *Carignan Noir* and *Tinta Amarela* were on the right side of PC1, disclosing a good correlation with seeds ABTS. However, the grape varieties *Bastardo*, *Gewürztraminer*, *Alvarilhão* and *Tinto Cão* were on the negative side of PC2, disclosing a good correlation with seeds DPPH.

A linear regression analysis was performed to determine the correlation between polyphenol composition and respective antioxidant activities in the different grape berry fractions. This analysis found no significant correlation ($p > 0.05$) between general phenolic parameters and antioxidant activity in the grape berry fractions. On the one hand, correlations between the antioxidant activity and the total polyphenolic content of a great number of grape seed and skin extracts from different grape varieties have been reported (Monagas *et al*., 2005; Xu *et al*., 2010). On the other hand, other authors (Bozan *et al*., 2008) reported no significant correlations between individual flavanols analyzed by HPLC or total polyphenols and antioxidant activity in seed extracts from several grape varieties. Thus, there is conflicting evidence in the literature about the correlation between polyphenol content and the antioxidant activity of grapes.

CONCLUSIONS

In this work, the influence of grape variety on the phenolic content, anthocyanin profile and antioxidant activity of the different grape berry fractions (skins, pulps and seeds) has been analyzed in twenty-four different grape varieties cultivated in two Portuguese wine regions (*Douro* and *Dão*). In general, a high variability was found among the diverse autochthonous and non-autochthonous grape varieties in relation to polyphenolic content, anthocyanin profile and antioxidant activity. Seeds were the grape berry fraction with the highest antioxidant activity, followed by skins and pulps, irrespective of the grape variety.

The results obtained for polyphenolic concentration, in particular individual anthocyanin concentration, and antioxidant activity must be considered in wineries, in order to apply the most appropriate winemaking techniques. Thus, from the data presented in this work, several specific winemaking techniques could be suggested depending on the grape variety: extended maceration could be used to optimise anthocyanin extraction and therefore improve color in *Bastardo* and *Alvarilhão* wines, whereas standard winemaking process could be a good option for *Carignan Noir*, *Tinta Miúda* and *Tinto Cão.* Increasing pumping over frequency during the maceration process can also increase the polyphenolic content and antioxidant capacity of wines, which is crucial for aging in oak barrels.

Finally, the variability found in phenolic content among grape varieties confirmed the hypothesis that genetic factors have an important role in the biosynthesis of these compounds and, consequently, in the antioxidant activity of grapes.

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